RESEARCH   **Open Access**

Transforming growth factor beta 1 levels predict echocardiographic changes at three years after adjuvant radiotherapy for breast cancer

Hanna Aula[1,2*] , Tanja Skyttä[1,2], Suvi Tuohinen[3,4], Tiina Luukkaala[5,6], Mari Hämäläinen[7], Vesa Virtanen[3], Pekka Raatikainen[4], Eeva Moilanen[7] and Pirkko-Liisa Kellokumpu-Lehtinen[1,2]

Abstract

Background: Transforming growth factor beta 1 (TGF-β1) and platelet-derived growth factor (PDGF) are cytokines involved in fibrotic processes causing radiotherapy (RT)-induced cardiovascular changes. We aimed to investigate the associations between TGF-β1 and PDGF and the echocardiographic changes that occur during RT and during three-year follow-up.

Methods: The study included 63 women receiving adjuvant RT for early-stage breast cancer or ductal carcinoma in situ. Serum TGF-β1 (ng/ml) and PDGF (ng/ml) levels were measured by enzyme-linked immunoassay and echocardiographic examination was performed before RT, after RT and at 3 years. Patients were grouped by biomarker behavior by a trajectory analysis.

Results: TGF-β1 decreased from 19.2 (IQR 17.1–22.3) before RT to 18.8 (14.5–22.0) after RT ($p = 0.003$) and the decrease persisted at 17.2 (13.7–21.2) 3 years after RT ($p = 0.101$). PDGF decreased from 15.4 (12.6–19.1) before RT to 13.8 (11.7–16.2) after RT, $p = 0.001$, and persisted at 15.6 (10.4–18.4) at 3 years, $p = 0.661$. The TGF-β1 level before RT (Spearman's rho 0.441, $p < 0.001$) and the three-year change in TGF-β1 (rho = − 0.302, $p = 0.018$) correlated with global longitudinal strain (GLS) in echocardiography at 3 years.

In trajectory analysis, two TGF-β1 behavior groups were found. Group 1 had significantly higher TGF-β1 levels before RT, 25.6 (22.3–28.6), than group 2, 17.8 (15.9–19.9), $p < 0.001$. In multivariable analysis, TGF-β1 trajectory group 1 (β = 0.27, $p = 0.013$), left-sided breast cancer (β = 0.39, $p = 0.001$) and the use of aromatase inhibitors (β = 0.29, $p = 0.011$) were significantly associated with a worsening in GLS from before RT to 3 years.

Conclusion: An elevated pretreatment TGF-β1 may predict RT-associated changes in echocardiography.

Keywords: Transforming growth factor beta 1, Platelet-derived growth factor, Cardiotoxicity, Breast cancer, Radiotherapy, Echocardiography

* Correspondence: hanna.aula@tuni.fi
[1]Faculty of Medicine and Health Technology, Tampere University, 33014
Tampere, Finland
[2]Department of Oncology, Tampere University Hospital, PO Box 2000, 33521
Tampere, Finland
Full list of author information is available at the end of the article

Aula *et al. Radiation Oncology*   (2019) 14:155

Page 2 of 9

Background

Adjuvant radiotherapy (RT) of breast cancer is associated with an increased risk of cardiovascular morbidity and mortality [1]. Transforming growth factor beta 1 (TGF-β1) is involved in the process responsible for the long-term fibrotic effects of RT, including cardiovascular changes and morbidity [2]. TGF-β1 is produced by platelets, macrophages, fibroblasts, monocytes, and epithelial and endothelial cells [2]. In a radiation fibrosis model, the production of profibrotic cytokines, such as TGF-β1 and platelet-derived growth factor (PDGF), is thought to be induced by radiation, which in turn activates fibroblasts. It is suggested that cytokines play an initiative role, but may not be necessary to maintain the fibrotic process [2].

TGF-β1 is also released following myocardial injury, exerting profibrotic actions on the myocardium. This process is involved in the pathogenesis of different myocardial diseases [3]. Elevated levels of TGF-β1 are also present in various fibrotic diseases, such as hepatic fibrosis, idiopathic pulmonary fibrosis, myelofibrosis or systemic sclerosis [4]. In addition, variation in circulating TGF-β1 levels is also caused by genetic factors [5]. Mutations in genes determining TGF-β1 levels may be responsible for susceptibility to radiation injury, such as breast fibrosis or increased cardiovascular morbidity [6, 7].

Only a few studies have examined the effect of adjuvant breast cancer RT on the circulating levels of TGF-β1. Two studies have reported that patients who developed fibrosis of the breast as sequelae of external beam RT had higher baseline levels of TGF-β1 than those who did not develop fibrosis [8, 9]. We reported that patients receiving adjuvant RT for breast cancer with echocardiographic changes from before to after RT had higher baseline TGF- β1 levels and their TGF-β1 and PDGF levels decreased during RT [10].

Our aim was to study the behavior of the TGF-β1 and PDGF levels and their association with the echocardiographic changes from before RT to immediately after and 3 years after RT.

Materials and methods

Patients

Altogether, 63 patients with available serum samples, receiving postoperative RT for breast cancer or ductal carcinoma in situ (DCIS) were included in this prospective, observational, single-center study. The included patients had breast-conserving surgery (n = 62) or a mastectomy (n = 1) before RT, but none received chemotherapy. Earlier publication describes the key inclusion and exclusion criteria in detail [11]. The Tampere University hospital ethics committee approved the study (R10160) and informed consent was obtained from all participants.

Radiotherapy

A detailed description of the RT protocol is found in an earlier publication [12]. Briefly, the planning target volume (PTV) received either 50 Gy in 2 Gy fractions or 42.56 Gy in 2.66 Gy fractions. The remaining breast after breast-conserving surgery or the chest wall after mastectomy with margins constituted the PTV. For the one patient with axillary node-positive disease, PTV included the axillary and supraclavicular areas.

Serum biomarker analysis

TGF-β1, PDGF and N-terminal pro-brain natriuretic peptide (proBNP) were analyzed from serum samples drawn at the start of RT, at the end of RT and 3 years after RT. Concentrations of TGF-β1 and PDGF-AB were measured by enzyme-linked immunosorbent assay with reagents from R&D Systems Europe Ltd. (Abingdon, UK). Samples were stored at − 80 °C and all samples were analyzed simultaneously with reagents from the same batch. Samples from before and after RT were also reanalyzed [10]. The detection limit and the interassay coefficient of variation were 7.8 ng/ml and 5.1% for TGF-β1 and 3.9 ng/ml and 3.5% for PDGF-AB, respectively.

Echocardiographic examination

Echocardiographic examination was performed at the same time points as when the serum samples were drawn. A single cardiologist (ST) performed all the examinations by a commercially available ultrasound machine (Philips iE33 ultrasound system; Philips, Bothell, WA, USA) and a 1–5 MHz matrix-array X5–1 transducer, as described previously [13, 14].

Statistical analysis

The median and interquartile range (IQR) were calculated for variables with skewed distributions. The Wilcoxon signed-rank test was used to test for change in a variable between measurements before RT, after RT and at 3 years. The linear relationship between continuous variables was determined using Spearman's correlation. The Mann-Whitney U-test for continuous variables was used to test for differences in the biomarkers, echocardiographic measurements or radiation doses between two groups. Group based trajectory modeling was used to determine the two trajectory groups [15]. The trajectory groups were created according to the three measurements of TGF-β1 or PDGF in each patient as a continuous outcome measure and the groups represent clusters of individuals with similar trajectories and outcomes over time [16]. Models were fitted by using the flexmix package [17] of the statistical program R, version 3.3.0, from the R Foundation for Statistical Computing [18]. The relative goodness of fit was assessed using Bayesian information criteria (BIC). Fischer's exact

Aula *et al. Radiation Oncology*   (2019) 14:155

Page 3 of 9

test was used to test for differences in categorical variables in the two trajectory groups. Linear regression was used to test multivariable associations with the change in GLS over 3 years. IBM SPSS Statistics software, version 25 for Windows (Armonk, NY, USA) was used for statistical testing. *P*-values under 0.05 were considered statistically significant.

Results

TGF-β1, PDGF and proBNP

Including all 63 patients, the median TGF-β1 decreased significantly during RT, $p = 0.003$ (Table 1). At the three-year follow-up, the median TGF-β1 level remained lower than before RT, $p = 0.001$. The median PDGF also decreased during RT, $p = 0.001$. The difference in median PDGF levels between before RT and at 3 years remained significant, $p = 0.046$. The median proBNP ($n = 62$) was stable during RT, $p = 0.325$, but increased by the three-year follow-up, $p < 0.001$.

The correlations of TGF-β1 and PDGF at corresponding time points and the changes between these time points are shown in Table 2. There were significant correlations between the TGF-β1 and PDGF as well as between the TGF-β1 and proBNP levels (Table 2), but PDGF and proBNP did not correlate together.

TGF-β1, PDGF and baseline characteristics

Age inversely correlated with the TGF-β1 level at 3 years (rho = − 0.310, $p = 0.013$) and the change in TGF-β1 from before RT to 3 years (rho = − 0.280, $p = 0.026$). There was no significant correlation between TGF-β1 and body mass index (BMI) or time from surgery to RT. Furthermore, there was no statistically significant difference in the TGF-β1 levels before RT in groups with different comorbidities or the use of medication, e.g. hypertension, hypothyrosis, coronary artery disease (CAD), smoking, diabetes and use of aromatase inhibitors (AI), tamoxifen, angiotensin convertase inhibitors (ACE), acetylsalicylic acid (ASA), or statins.

PDGF at 3 years also inversely correlated with age (rho = − 0.348, $p = 0.005$), but not with BMI or time from surgery to RT. Statin-users had lower median PDGF levels before RT than did nonusers, 12.0 (10.2–16.5) ng/ml and 16.5 (13.4–20.4) ng/ml, respectively ($p = 0.024$). Furthermore, tamoxifen-users had lower median PDGF levels after RT than nonusers did, 12.1 (11.6–17.3) ng/ml and 15.6 (12.8–20.1) ng/ml, respectively ($p = 0.041$). There were no differences in the median PDGF levels according to the other baseline characteristics.

TGF-β1 and PDGF levels and echocardiographic measurements

The TGF-β1 and PDGF levels before RT, after RT, at 3 years, and the changes between these time points correlated with different structural and functional parameters in echocardiography. Echocardiographic measurements are presented in Additional file 1: Table S1. The significant correlations for TGF-β1 and PDGF after RT and at 3 years and echocardiography are presented in Additional file 2: Table S2.

Correlations with structural echocardiographic measurement

The interventricular septum (IVS) (rho 0.256, $p = 0.042$) and posterior wall (PW) (rho = 0.318, $p = 0.011$) thicknesses and the left ventricular end systolic diameter (LVESD) (rho 0.300, $p = 0.017$) after RT correlated with TGF-β1 before RT. Additionally, the IVS (rho 0.428, $p < 0.001$) and PW (rho = 0.389, $p = 0.002$) thicknesses at 3 years correlated with the TGF-β1 level before RT.

Furthermore, the change in TGF-β1 during RT inversely correlated with the change in septal calibrated integrated backscatter (scIBS) during RT (rho = −0.289, $p = 0.023$). The change in TGF-β1 from before RT to 3 years inversely correlated with the change in IVS from before RT to 3 years (rho = −0.255, $p = 0.044$) and the IVS at 3 years (rho = −0.383, $p = 0.002$).

The PDGF level before RT correlated with the change in IVS from before RT to 3 years (rho = 0.306, $p = 0.015$). The PDGF change during RT correlated inversely with the posterior calibrated integrated backscatter (pcIBS) before RT (rho = −0.341, $p = 0.007$) and positively with the change in pcIBS during RT (rho = 0.307, $p = 0.016$) and the change in pcIBS from baseline to 3 years (rho = 0.336, $p = 0.009$). The PDGF change from before RT to 3 years correlated positively with the change in left ventricular end diastolic diameter (LVEDD) (rho = 0.267, $p = 0.035$) and negatively with the change in IVS (rho = −0.385, $p = 0.002$) from before RT to 3 years.

Table 1 TGF-β1, PDGF and proBNP levels in the whole study population

| | n | Before RT | | After RT | | Three years | | p^1 | p^2 | p^3 |
		Md	IQR	Md	IQR	Md	IQR			
TGF-β1 (ng/ml)	63	19.2	(17.1–22.3)	18.8	(14.5–22.0)	17.2	(13.7–21.2)	**0.003**	**0.001**	0.101
PDGF (ng/ml)	63	15.4	(12.6–19.1)	13.8	(11.7–16.2)	15.6	(10.4–18.4)	**0.001**	**0.046**	0.661
proBNP (ng/l)	62	75.5	(42.5–125.5)	75.0	(35.5–132.8)	101.0	(55.5–183.3)	0.325	**< 0.001**	**< 0.001**

TGF-β1 Transforming growth factor beta 1, *PDGF* Platelet-derived growth factor, *proBNP* N-terminal pro-brain natriuretic peptide, *RT* Radiotherapy, *Md* Median, *IQR* Interquartile range, p^1 Change from before to after RT, p^2 Change from before to 3 years after RT, p^3 Change from after RT to 3 years after RT

Aula *et al. Radiation Oncology*   (2019) 14:155

Page 4 of 9

Table 2 Correlations between TGF-β1, PDGF and proBNP

	TGF-β1 before RT	TGF-β1 after RT	TGF-β1 3 years	Change in TGF-β1 before RT–after RT	Change in TGF-β1 before RT–3 years	Change in TGF-β1 after RT–3 years
PDGF before RT	**0.667**	0.222	0.132	**—0.405**	**—0.480**	—0.088
PDGF after RT	**0.453**	**0.713**	**0.316**	**0.249**	—0.141	**—0.361**
PDGF 3 years	**0.299**	**0.338**	**0.749**	0.022	**0.336**	**0.414**
Change in PDGF before RT–after RT	—0.221	**0.481**	0.246	**0.646**	**0.363**	—0.204
Change in PDGF before RT–3 years	**—0.254**	—0.128	**0.625**	0.308	**0.739**	**0.513**
Change in PDGF after RT–3 years	—0.132	**—0.267**	**0.544**	—0.152	**0.600**	**0.826**
proBNP before RT	**0.298**	—0.037	—0.040	**—0.325**	**—0.280**	—0.050
proBNP after RT	**0.265**	—0.041	—0.006	**—0.252**	—0.183	—0.005
proBNP 3 years	**0.293**	—0.060	—0.061	**—0.321**	**—0.297**	—0.070
Change in proBNP before RT–after RT	—0.040	—0.016	0.054	0.101	0.150	0.075
Change in proBNP before RT–3 years	0.114	0.087	0.004	—0.023	—0.111	—0.129
Change in proBNP after RT–3 years	0.212	0.018	0.004	**—0.259**	—0.232	—0.060

TGF-β1 transforming growth factor beta 1, *PDGF* Platelet-derived growth factor, *proBNP* N-terminal pro-brain natriuretic peptide, *RT* Radiotherapy, Correlation coefficients (Spearman's rho) in bold are statistically significant ($p < 0.05$)

Correlations with systolic echocardiographic measurements

The global longitudinal strain (GLS) at 3 years correlated positively with the TGF-β1 level before RT (rho 0.441, $p < 0.001$) and inversely with the TGF-β1 change during RT (rho = —0.302, $p = 0.018$). Furthermore, the GLS change from before RT to 3 years inversely correlated with the PDGF level before RT (rho = —0.288, $p = 0.022$). Likewise, the PDGF change during RT correlated with the TAPSE change from before RT to 3 years (rho = 0.262, $p = 0.045$).

Correlations with measurements of filling pressure and diastology in echocardiography

The TGF-β1 level before RT inversely correlated with the mitral early inflow wave velocity (mitral E) (rho —0.300, $p = 0.017$) after RT. The PDGF level before RT inversely correlated with the mitral E before RT (rho = —0.288, $p = 0.022$), after RT (rho = —0.416, $p = 0.001$) and at three years (rho = —0.270, $p = 0.033$).

TGF-β1 trajectories

Trajectory analysis was performed to group the patients by behavior of TGF-β1. Group 1 ($n = 19$) had significantly higher levels of TGF-β1 before RT, after RT and at 3 years than group 2 ($n = 44$), $p < 0.001$ for all time points (Table 3). In group 1, there was a tendency for TGF-β1 levels to decrease from before to after RT and to the three-year follow-up, $p = 0.066$ and $p = 0.080$ respectively. In group 2, there was a significant decrease in the TGF-β1 level from baseline to after RT, $p = 0.023$, and to the three-year follow-up, $p = 0.006$. The groups were similar in baseline characteristics (Table 3), and there were no significant differences in the proBNP levels at the three time points between the groups.

Echocardiographic parameters by the two trajectory groups are shown in Table 4. Baseline measurements were similar between the two groups. The IVS at 3 years, the PW after RT and the PW at 3 years were significantly different between the groups, $p = 0.016$, $p = 0.039$ and $p = 0.010$, respectively. There was a tendency for a difference in GLS at 3 years, $p = 0.081$. During RT, there was a significant change for group 1 in IVS ($p = 0.036$), PW ($p = 0.030$), TAPSE ($p = 0.021$) and scIBS ($p = 0.030$). For group 2 there was a significant change during RT in TAPSE ($p = 0.030$) and scIBS ($p = 0.007$). During the three-year follow-up, GLS and scIBS significantly worsened from baseline in group 1, $p = 0.013$ and $p < 0.001$, respectively. In group 2, PW decreased and scIBS increased from baseline to 3 years, $p = 0.028$ and $p = 0.002$, respectively. Radiation doses to the heart, left ventricle (LV), right ventricle (RV) and the left anterior descending artery (LAD) were similar between the two trajectory groups (Additional file 3: Table S3).

To further explore the association between TGF-β1 and GLS suggested by correlation and the significant worsening in trajectory group 1, multivariable linear regression analysis was performed. In the model, TGF-β1 trajectory group 1 (β = 0.27, p = 0.013), left-sided breast cancer (β = 0.39, $p = 0.001$) and the use of AI (β = 0.29, $p = 0.011$) were significantly associated with a reduction in GLS from before RT to 3 years. Additionally, there was tendency for age to be associated (β = 0.18, $p = 0.071$) with worsening GLS during the three-year follow-up. These factors explained 33% of the change in GLS.

PDGF trajectories

A trajectory analysis was also performed for PDGF. PDGF levels were significantly higher at all time-points

Aula *et al. Radiation Oncology*   (2019) 14:155

Page 5 of 9

Table 3 TGF-β1 levels and baseline characteristics according to two groups determined by a TGF-β1 trajectory analysis

	n	Group 1	n	Group 2	p
TGF-β1 before RT (ng/ml) Md (IQR)	19	25.6 (22.3–28.6)	44	17.8 (15.9–19.9)	**< 0.001**
TGF-β1 after RT (ng/ml), Md (IQR)	19	23.9 (21.3–25.2)	44	16.3 (13.7–19.1)	**< 0.001**
TGF-β1 at 3 years (ng/ml), Md (IQR)	19	22.4 (18.8–25.8)	44	15.0 (12.1–18.8)	**< 0.001**
Age, Md (IQR)	19	64 (58–66)	44	64 (59–68)	0.752
BMI, Md (IQR)	19	26.9 (24.8–32.3)	44	25.7 (24.1–28.7)	0.110
Left-sided BC, n (%)	19	14 (73.7)	44	36 (81.8)	0.508
AI-use, n (%)	19	6 (31.2)	44	15 (34.1)	1.000
Tamoxifen use, n (%)	19	2 (10.5)	44	4 (9.1)	1.000
ACE or ARB use, n (%)	19	5 (26.3)	44	12 (27.3)	1.000
ASA use, n (%)	19	1 (5.3)	44	4 (9.1)	1.000
Beta-blocker use, n (%)	19	4 (21.1)	44	7 (15.9)	0.721
Statin use, n (%)	19	3 (15.8)	44	10 (22.7)	0.738
CAD, n (%)	19	2 (10.5)	44	1 (2.3)	0.214
Diabetes, n (%)	17	3 (17.6)	42	2 (4.8)	0.138
Hypertension, n (%)	19	9 (47.4)	44	15 (34.1)	0.400
Hypothyroidism, n (%)	19	3 (15.8)	44	7 (15.9)	1.000
Smoking, n (%)	19	2 (10.5)	44	5 (11.4)	1.000

TGF-β1 Transforming growth factor beta 1, *RT* Radiotherapy, *Md* Median, *IQR* Interquartile range, *BMI* Body mass index, *BC* Breast cancer, *AI* Aromatase inhibitor, *ACE* Angiotensin converting enzyme inhibitor, *ARB* Angiotensin II receptor blocker, *ASA* Low dose acetylsalicylic acid, *CAD* Coronary artery disease, *Diabetes* Use of diabetes medication

in group 1 ($n = 8$) than in group 2 ($n = 55$), $p < 0.001$ (Additional file 4: Table S4) for all time-points. The groups did not differ in baseline characteristics (Additional file 4: Table S4). The change in PDGF was only significant in group 2 from before to after RT, $p = 0.001$.

Only scIBS at 3 years was significantly higher in group 1 than group 2, $p = 0.044$. The elevated PDGF levels in group 1 were not associated with more changes in echocardiographic parameters, but the group 1 was too small for a meaningful comparison (Additional file 5: Table S5). Furthermore, radiation doses to the heart, LV, RV or LAD were similar in the groups (Additional file 4: Table S4).

Discussion
Elevated baseline TGF-β1 associates with echocardiographic changes
The most important finding in our study was the association of elevated TGF-β1 before RT with a decline in LV systolic function, namely, impairment in GLS during the three-year follow-up. This association was apparent in the correlation between TGF-β1 and GLS at 3 years and further with the trajectory analysis in which patients were grouped into two groups according to TGF-β1 behavior. Group 1 had significantly higher baseline TGF-β1 levels than group 2. At baseline, the echocardiographic parameters were similar, but RT induced a thickening of the IVS and PW during RT in group 1, but not in group 2. These changes most likely depict RT-

induced inflammatory changes. During the three-year follow-up, group 1 had a significant worsening of GLS and group 2 did not. In multivariable analysis, the trajectory group 1 remained a significant factor in predicting GLS worsening during the 3 years in addition to AI use and left-sided breast cancer, which we have reported in earlier to affect echocardiographic parameters [12, 13]. Left-sided breast cancer associates with higher radiation doses to the heart [19], which probably explains the significance of the side. Radiation dose is a significant factor determining cardiovascular risk of breast cancer patients and dose-volume constraints are in clinical use to decrease this risk [20]. Radiation doses were similar between the groups, as the grouping mostly reflects a difference in baseline TGF-β1. The worsening in GLS is probably the most clinically significant echocardiographic change, as GLS is an excellent early predictor of major adverse cardiac events [21]. Additionally, the correlation between the levels of TGF-β1 and proBNP, an accepted marker of heart failure [22], at all three time-points further supports the association of elevated TGF-β1 levels and cardiovascular changes.

In previous literature, two other studies with adjuvant external beam RT [8] and intracavitary partial breast brachytherapy [9] reported elevated circulating baseline TGF-β1 levels in patients who developed radiation-induced fibrosis of the breast. Studies with genetic mutations also support the idea that baseline TGF-β1 levels may predispose individuals to normal tissue toxicity

Table 4 Echocardiographic measurements according to TGF-β1 trajectory groups

	Group 1 (n = 19)								Group 2 (n = 44)								
	Before RT		After RT		3 year		p¹	p²	Before RT			After RT		3 year		p¹	p²
	n	Md	IQR	Md	IQR	Md	IQR			n	Md	IQR	Md	IQR	Md	IQR		
LV measurements																		
LVEDD (mm)	19	46.0	(44.0–48.0)	45.0	(44.0–50.0)	46.0	(42.0–50.0)	0.513	0.446	44	46.0	(44.0–47.0)	46.0	(43.3–47.0)	45.0	(43.0–47.0)	0.904	0.654
LVESD (mm)	19	31.0	(29.0–34.0)	32.0	(28.0–34.0)	31.0	(29.0–35.0)	0.854	0.954	44	31.0	(29.0–32.8)	30.5	(29.0–31.8)	30.0	(29.0–32.0)	0.781	0.896
IVS (mm)	19	10.0	(9.0–11.0)	11.0	(10.0–12.0)	11.0	(10.0–11.0)	**0.036**	0.157	44	10.0	(9.0–11.0)	10.0	(9.0–11.0)	10.0	(9.0–11.0)	0.378	0.775
PW (mm)	19	10.0	(9.0–11.0)	11.0	(10.0–12.0)	9.0	(10.0–11.0)	**0.030**	0.434	44	10.0	(9.0–11.0)	10.0	(9.3–11.0)	9.0	(8.3–10.0)	0.144	**0.028**
LV systolic function																		
LV EF (%)	19	62.0	(56.0–65.0)	61.0	(58.0–65.0)	60.0	(57.0–63.0)	0.886	0.144	44	62.0	(60.0–65.0)	63	(59.3–65.0)	61.5	(57.0–63.0)	1.000	0.092
GLS (%)	17	−17.0	(−20.5−16.0)	−17.0	(−19.5−15.5)	−16.0	(−18.5−14.0)	0.373	**0.006**	44	−18.0	(−19.8−15.0)	−17.0	(−20.0−15.0)	−18.0	(−20.0−16.0)	0.159	0.762
LV diastolic function																		
Mitral inflow E (cm/s)	19	73.5	(66.2–81.9)	66.1	(57.2–79.0)	66.2	(61.7–78.4)	0.113	0.106	44	75.4	(63.8–87.3)	72.4	(60.3–80.0)	71.4	(62.0–89.0)	0.139	0.242
Ee' ratio	19	8.3	(6.8–10.2)	7.8	(7.1–11.1)	8.7	(7.1–10.2)	0.701	0.347	44	9.5	(7.7–11.4)	9.3	(7.1–10.2)	8.9	(7.7–11.0)	0.076	0.337
RV function																		
TAPSE (mm)	17	24.0	(21.0–28.0)	21.0	(18.5–24.5)	22.0	(20.5–27.5)	**0.021**	0.363	42	24.5	(20.8–27.3)	22.0	(19.0–26.0)	24.0	(20.0–26.0)	**0.001**	0.119
TR gradient (mmHg)	12	21.0	(18.3–26.0)	20.5	(17.3–24.0)	23.5	(19.3–28.5)	0.351	0.381	35	21.0	(17.0–25.0)	22.0	(19.0–24.0)	24.0	(20.0–29.0)	0.811	**< 0.001**
Tissue characterization																		
scIBS (dB)	17	15.5	(12.5–21.3)	19.5	(13.7–25.1)	23.3	(20.1–25.8)	**0.031**	**< 0.001**	44	17.6	(14.1–22.1)	19.6	(16.3–24.2)	20.3	(18.8–22.8)	**0.007**	**0.002**
rcIBS (dB)	17	18.7	(14.8–21.8)	22.9	(20.1–25.9)	23.5	(19.1–27.0)	**0.011**	**0.013**	44	21.1	(17.1–24.6)	23.0	(18.0–26.9)	24.1	(20.3–27.0)	0.208	**0.021**
pcIBS (dB)	17	10.3	(7.6–11.9)	9.2	(8.2–12.1)	9.4	(6.5–13.1)	0.579	0.854	43	9.8	(6.8–13.9)	10.3	(8.3–12.5)	10.5	(6.8–13.4)	0.888	0.959

TGF-β1 Transforming growth factor beta 1, *RT* Radiotherapy, *Md* Median, *IQR* Interquartile range, *p¹* p-value for before to after RT, *p²* p-value for before to 3 years after RT, *LV* Left ventricle, *LVEDD* Left ventricle end diastolic diameter, *LVESD* Left ventricle end systolic diameter, *IVS* Interventricular septum thickness, *PW* Posterior wall thickness, *EF* Ejection fraction, *GLS* Global longitudinal strain, *Mitral inflow E* First peak of diastole, *Ee'* Pulsed tissue doppler e' velocity, *RV* Right ventricle, *TAPSE* Tricuspid annular plane systolic excursion, *TR gradient* Tricuspid regurgitation maximal gradient, *scIBS* Septal calibrated integrated backscatter, *rcIBS* Right ventricle integrated backscatter, *pcIBS* Posterior wall of left ventricle integrated backscatter

from RT. Circulating levels of TGF-β1 are, at least partially, controlled genetically [3]. The TGF-β1 C-509 T variant allele (rs1800469) is associated with elevated levels of circulating TGF-β1 [3]. Prospective studies present contradictory evidence of the association between TGF-β1 C-509 T variant allele and radiation-induced fibrosis of the breast [4, 5]. There is some evidence that mutations in TGF-β1 genes are linked to cardiovascular risk. In patients receiving postoperative RT for breast cancer, patients with the TGF-β1 29C > T variant allele, associated with low TGF-β1 levels, had an increased cardiovascular risk with HR 1.79. However, in this study, there was no association between CV mortality and radiation dose [7]. Furthermore, the role of TGF-β1 is not clear in cardiovascular diseases. The evidence on the role of TGF-β1 in atherosclerosis is contradictory, but most studies suggest TGF-β1 inhibits atherosclerosis [23]. In contrast, elevated levels of TGF-β1 are associated with, for example, hypertrophic cardiomyopathy [23].

Changes in TGF-β1 levels

Earlier, we reported that TGF-β1 and PDGF decrease during RT [10], but this study shows that TGF-β1 remains at a lower level at 3 years compared to before RT. The decreases were small but statistically significant. In a suggested model, RT is thought to induce a cytokine release [2], but since we only have measurements for before and after RT, a cytokine release could have taken place earlier during the RT course. One previous study with intraoperative RT (IORT) for breast cancer reported that IORT had no effect on the TGF-β1 levels measured from wound fluid [24]. We found that a decrease in TGF-β1 during RT and the three-year follow-up correlated with worsening LV systolic function e.g. GLS. Additionally, the three-year change was correlated with an increased echodensity of the myocardium, scIBS, and LV measurements. The inverse correlations between the change in TGF-β1 levels and proBNP levels, further suggest that the decrease in TGF-β1 may be associated with an increased cardiovascular risk.

PDGF levels and associations with echocardiographic measurements

We also found associations between elevated baseline PDGF levels and LV systolic function, e.g. worsening GLS, and LV diastolic function, e.g. decreased mitral E. The change in PDGF during RT also predicted impairment in GLS over 3 years. The trajectory analysis did not further support the usability of PDGF in predicting RT-induced echocardiographic changes. Additionally, there were no correlations between PDGF and proBNP. Therefore, despite the strong correlation between the two biomarkers, TGF-β1 seems to be more usable than PDGF in predicting the cardiovascular effects of RT. To

our knowledge, no previous studies exist about the PDGF and RT-induced toxicity in humans.

Limitations

Although we now present results with 3 years of follow-up, the follow-up time is still short, considering that the increased risk of cardiovascular effects of RT takes years to manifest. Longer follow-up will show whether the echocardiographic changes lead to clinical cardiovascular morbidity. This may require larger studies than ours. Additionally, the underlying cause of elevation of TGF-β1 is not known nor do we have information on how well other risk factors of cardiovascular diseases, such as elevated blood pressure, diabetes, and hyperlipidemia, are controlled in the patients. These factors could influence the results.

Conclusions

Our finding supports that elevated TGF-β1 before RT is a risk factor for the susceptibility to normal tissue toxicity and is associated with RT-induced changes in echocardiography, namely, impairment in GLS, a measure of LV systolic function. An elevated TGF-β1 level is an independent risk factor for the impairment in GLS in addition to AI use and left-sided breast cancer, which is likely due to higher radiation doses in left-sided breast cancer. Furthermore, a decrease in TGF-β1 during the three-year follow-up also correlated with echocardiographic changes. More studies and longer follow-up are needed to confirm whether elevated TGF-β1 can be used to determine which patients are at an increased risk for radiation-induced heart disease. Further, this information could even be useful when designing future cardioprotective trials.

Additional files

Additional file 1: Table S1. Echocardiographic measurements in the whole study population. (DOCX 33 kb)

Additional file 2: Table S2. Echocardiographic measurements with significant correlations with TGF-β1 and PDGF after RT and at 3 years. (DOCX 30 kb)

Additional file 3: Table S3. Radiation doses to the heart according to groups determined by TGF-β1 trajectory analysis. (DOCX 31 kb)

Additional file 4: Table S4. PDGF levels and baseline characteristics according to groups determined by PDGF trajectory analysis. (DOCX 33 kb)

Additional file 5: Table S5. Echocardiographic measurements according to PDGF trajectory groups. (DOCX 38 kb)

Abbreviations

ACE: Angiotensin converting enzyme inhibitor; AI: Aromatase inhibitor; ARB: Angiotensin II receptor blocker; ASA: Low dose acetylsalicylic acid; BMI: Body mass index; CAD: Coronary artery disease; DCIS: Ductal carcinoma in situ; Ee′: Pulsed tissue doppler e′ velocity; EF: Ejection fraction; GLS: Global longitudinal strain; IQR: Interquartile range; IVS: Interventricular septum thickness; LV: Left ventricle; LVEDD: Left ventricle end diastolic diameter;

Aula *et al. Radiation Oncology*   (2019) 14:155

Page 8 of 9

LVESD: Left ventricle end systolic diameter; Md: Median; Mitral inflow E: First peak of diastole; pcIBS: Posterior wall of left ventricle integrated backscatter; PDGF: Platelet-derived growth factor; proBNP: N-terminal pro-brain natriuretic peptide; PTV: Planning target volume; PW: Posterior wall thickness; rcIBS: Right ventricle integrated backscatter; RT: Radiotherapy; RV: Right ventricle; scIBS: Septal calibrated integrated backscatter; TAPSE: Tricuspid annular plane systolic excursion; TGF-β1: Transforming growth factor beta 1; TR gradient: Tricuspid regurgitation maximal gradient

Authors' contributions
HA prepared the draft of the manuscript and all other authors provided critical revisions of the content. HA, TS, ST, MH, VV, PR, EM and PK contributed to the conception or design of the study. MH and EM were responsible for the analysis of the samples. HA and TL performed and interpreted statistical analyses. All authors read and approved the final manuscript.

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The Tampere University hospital ethics committee approved the study (R10160) and informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Faculty of Medicine and Health Technology, Tampere University, 33014 Tampere, Finland. [2]Department of Oncology, Tampere University Hospital, PO Box 2000, 33521 Tampere, Finland. [3]Heart Hospital, Tampere University Hospital, PO Box 2000, 33521 Tampere, Finland. [4]Department of Cardiology, Heart and Lung Center, Helsinki University Hospital, PO Box 340, 00029 HUS Helsinki, Finland. [5]Research, Innovation and Development Center, Tampere University Hospital, PO Box 2000, 33521 Tampere, Finland. [6]Health Sciences, Faculty of Social Sciences, Tampere University, 33014 Tampere, Finland. [7]The Immunopharmacology Research Group, Faculty of Medicine and Health Technology, Tampere University and Tampere University Hospital, 33014 Tampere, Finland.

References
1. Cheng Y, Nie X, Ji C, Lin X, Liu L, Chen X, et al. Long-term cardiovascular risk after radiotherapy in women with breast Cancer. J Am Heart Assoc. 2017;6. https://doi.org/10.1161/JAHA.117.005633.
2. Yarnold J, Vozenin Brotons M-C. Pathogenetic mechanisms in radiation fibrosis. Radiother Oncol. 2010;97:149–61. https://doi.org/10.1016/j.radonc.2010.09.002.
3. Frangogiannis NG. Cardiac fibrosis: cell biological mechanisms, molecular pathways and therapeutic opportunities. Mol Asp Med. 2018. https://doi.org/10.1016/j.mam.2018.07.001.
4. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor β in human disease. N Engl J Med. 2000;342:1350–8. https://doi.org/10.1056/NEJM200005043421807.
5. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet. 1999;8:93–7.
6. Grossberg AJ, Lei X, Xu T, Shaitelman SF, Hoffman KE, Bloom ES, et al. Association of Transforming Growth Factor β polymorphism C−509T with radiation-induced fibrosis among patients with early-stage breast Cancer. JAMA Oncol. 2018. https://doi.org/10.1001/jamaoncol.2018.2583.
7. Hilbers FSM, Boekel NB, van den Broek AJ, van Hien R, Cornelissen S, Aleman BMP, et al. Genetic variants in TGFβ-1 and PAI-1 as possible risk factors for cardiovascular disease after radiotherapy for breast cancer. Radiother Oncol. 2012;102:115–21. https://doi.org/10.1016/j.radonc.2011.10.017.
8. Li C, Wilson PB, Levine E, Barber J, Stewart AL, Kumar S. TGF-beta1 levels in pre-treatment plasma identify breast cancer patients at risk of developing post-radiotherapy fibrosis. Int J Cancer. 1999;84:155–9.
9. Boothe DL, Coplowitz S, Greenwood E, Barney CL, Christos PJ, Parashar B, et al. Transforming growth factor β-1 (TGF-β1) is a serum biomarker of radiation induced fibrosis in patients treated with Intracavitary accelerated partial breast irradiation: preliminary results of a prospective study. Int J Radiat Oncol Biol Phys. 2013;87:1030–6. https://doi.org/10.1016/j.ijrobp.2013.08.045.
10. Aula H, Skyttä T, Tuohinen S, Luukkaala T, Hämäläinen M, Virtanen V, et al. Decreases in TGF-β1 and PDGF levels are associated with echocardiographic changes during adjuvant radiotherapy for breast cancer. Radiat Oncol. 2018;13:201. https://doi.org/10.1186/s13014-018-1150-7.
11. Tuohinen SS, Skytta T, Virtanen V, Luukkaala T, Kellokumpu-Lehtinen P-L, Raatikainen P. Early effects of adjuvant breast cancer radiotherapy on right ventricular systolic and diastolic function. Anticancer Res. 2015;35:2141–7.
12. Skytta T, Tuohinen S, Virtanen V, Raatikainen P, Kellokumpu-Lehtinen P-L. The concurrent use of aromatase inhibitors and radiotherapy induces echocardiographic changes in patients with breast cancer. Anticancer Res. 2015;35:1559–66.
13. Tuohinen SS, Skytta T, Poutanen T, Huhtala H, Virtanen V, Kellokumpu-Lehtinen P-L, et al. Radiotherapy-induced global and regional differences in early-stage left-sided versus right-sided breast cancer patients: speckle tracking echocardiography study. Int J Cardiovasc Imaging. 2017;33:463–72. https://doi.org/10.1007/s10554-016-1021-y.
14. Tuohinen SS, Skytta T, Virtanen V, Virtanen M, Luukkaala T, Kellokumpu-Lehtinen P-L, et al. Detection of radiotherapy-induced myocardial changes by ultrasound tissue characterisation in patients with breast cancer. Int J Cardiovasc Imaging. 2016;32:767–76. https://doi.org/10.1007/s10554-016-0837-9.
15. Nagin DS. Group-based modeling of development. Cambridge: Harvard Univesity Press; 2005. https://doi.org/10.4159/9780674041318.
16. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. Annu Rev Clin Psychol. 2010;6:109–38. https://doi.org/10.1146/annurev.clinpsy.121208.131413.
17. Leisch F. FlexMix: A general framework for finite mixture models and latent glass regression in R, vol. 11; 2004.
18. R core team. R: a language and environment for statistical computing 2018.
19. Taylor CW, Nisbet A, McGale P, Darby SC. Cardiac exposures in breast Cancer radiotherapy: 1950s–1990s. Int J Radiat Oncol. 2007;69:1484–95. https://doi.org/10.1016/j.ijrobp.2007.05.034.
20. Gagliardi G, Constine LS, Moiseenko V, Correa C, Pierce LJ, Allen AM, et al. Radiation dose–volume effects in the heart. Int J Radiat Oncol. 2010;76:S77–85. https://doi.org/10.1016/j.ijrobp.2009.04.093.
21. Kalam K, Otahal P, Marwick TH. Prognostic implications of global LV dysfunction: a systematic review and meta-analysis of global longitudinal strain and ejection fraction. Heart. 2014;100:1673–80. doi. https://doi.org/10.1136/heartjnl-2014-305538.
22. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Colvin MM, et al. 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the Management of Heart Failure: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines and the heart failure Society of Amer. Circulation. 2017;136:e137–61. https://doi.org/10.1161/CIR.0000000000000509.

23. Gordon KJ, Blobe GC. Role of transforming growth factor-β superfamily signaling pathways in human disease. Biochim Biophys Acta - Mol Basis Dis. 2008;1782:197–228. https://doi.org/10.1016/j.bbadis.2008.01.006.

24. Scherer SD, Bauer J, Schmaus A, Neumaier C, Herskind C, Veldwijk MR, et al. TGF-β1 is present at high levels in wound fluid from breast Cancer patients immediately post-surgery, and is not increased by intraoperative radiation therapy (IORT). PLoS One. 2016;11:e0162221. https://doi.org/10.1371/journal.pone.0162221.

Publisher's Note